183 Cytotoxic activity evidence of *Achromobacter xylosoxidans* for pulmonar tissue

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Achromobacter xylosoxidans is a non-glicose-fermenting environmental Gram-negative rod with increasing recovering frequency from the respiratory tract of Cystic Fibrosis (CF) patients. The pathogenic role of this microorganism is still unclear including the knowledge of its virulence factors. We are searching for potencial virulence factors in *A. xylosoxidans* strains isolated from CF pediatric patients seen at the outpatient reference center at our State University Hospital. The strains were identified by conventional tests, by automated Vitek II® and controlled with LMG® reference strains and by PCR. The culture supernatants in BHI of 10 different clinical isolates of *A. xylosoxidans* and the LMG 1863 strain were assayed for cytotoxic activity. Cytotoxic activity was observed only in H-460 (human lung carcinoma), inducing blebbings and cellular death after 18 hours assays for all strains. Microscopic analysis revealed rounding and membrane alterations followed by detachment and cellular death. However no cytotoxic activity was verified on HeLa (human cervix carcinoma), 3T3 (mouse fibroblast) and Vero (green monkey kidney) cells. These results suggest that the cytotoxic activity of *A. xylosoxidans* to human lung cells (H-460) could be a potencial mechanism of pathogenicity that may participate in the process of lung parenchyma destruction in cystic fibrosis patients and should be better investigated.

184* Treatment of Non-Tuberculous Mycobacteria in Adults with Cystic Fibrosis

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We undertook a 4 year retrospective analysis of patients attending Papworth Hospital Adult Cystic Fibrosis Unit from 2004–8.

Sputum surveillance for Non-Tuberculous Mycobacteria (NTM) was undertaken at least annually and additionally when directed by abnormalities on CT scans or if patients failed to respond to conventional antibiotics.

Between 2004–8 the annual incidence of new NTM positive sputum cultures increased successively from 1.4% (3/217) in 2004–5, 2.3% (5/221) in 2005–6, 3.1% (7/228) in 2006–7 and 4.3% (10/230) in 2007–8. Over this period, the most frequently isolated species were M abscessus/chelonae (10/25), M avium/intracellulare (5/25) and M kansasii (3/25). 10 patients during this period were diagnosed with NTM disease of which 8 had M abscessus.

We have developed a treatment algorithm for M abscessus involving 2–3 weeks induction therapy with 3/4 intravenous antibiotics followed by maintenance therapy with 3/4 oral and 1/2 nebulised antibiotics with or without subcutaneous interferon gamma. Exacerbations are treated with additional intravenous antibiotics for 2–3 weeks followed by augmentation of maintenance therapy.

A total of 12 patients have been treated for M abscessus/chelonae at our unit: 4 remain NTM free after >6 months of stopping treatment; 4 are on treatment and have been culture negative for >6 months while 4 patients continue to culture M abscessus despite maximum medical therapy.

We conclude that the incidence of NTM disease is increasing in adults with Cystic Fibrosis, that M abscessus is the major pathgenen and that successful treatment is possible in at least some patients.

185 Chronic bronchial infection (cbi) with Bordetella bronchiseptica (Bb) in CF

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Bb is an aerobic gram(–) organism, causing highly transmissible acute respiratory infections in mammals. Acute respiratory infections in humans – often immunocompromised – have been rarely reported. Nearly all had close contact with animals. Cbi with Bb has been reported only once in CF

We have had 5 cases of cbi with Bb out of 170 CF patients (2.9%). Strains were sent to a reference lab, which confirmed the isolates, and studied them with pulsed field gel electrophoresis (PGFE). All were male aged 12–25 mean 15.5 years. None was immunocompromised and only one had close contact with animals (dogs and cats).PGFE showed that 4 (2 siblings) shared the same genetic pattern but except in the two siblings, there was no contact among them. All had minimal or mild pulmonary disease, and except the oldest one all had long cough-freee periods. None had cbi with other organism. They all developed cbi with Bb only, except the oldest one who also had intermitent *S. aureus* isolations. Three had no clinical impairment, but both siblings developed daily cough shortly after colonization and had a quick worsening in their clinical symptoms, chest X-ray, and pulmonary function. FEV1 (% Pred)fell from 99% to 57% over 5 years in the older and from 100% to 78% over 2 years in the younger one.A poor correlation between antibiotic sensitivity reports and clinical response was observed. Bb is an emerging organism in CF which can cause cbi. It is potentially transmissible and may be asociated with a steep clinical decline in some patients. We think that every patient in whom Bb is grown should be isolated from other CF patients to prevent crossinfection.

186 Clinical follow-up of 13 French CF patients with *Inquilinus limosus*

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Inquilinus limosus (IL) is a recently described mucoid multiresistant Gram-negative non fermenting species in the sputum of CF airways. Data were collected from all French CF centres by the French Observatoire cepacia.

From 2002 to 2007, 15 colonizations were reported in 9 CF Centres. IL was recovered from a Burkholderia cepacia selective medium and identified by means of 16S rRNA analysis. The mean age at primocolonization was 11.6 (2–21). 7/15 colonizations were chronic, and the mean duration of the follow-up in these patients was 3.8 (2–6) years. Of the 7 chronically colonized patients, 1 deceased 2 years after the primocolonization and IL was strongly incriminated, 4 remain stable, and 2 deteriorated. Eradication of IL was unsuccessful despite several imipenem courses. In conclusion, IL is increasingly recognized in CF, due to improved recovery and identification of this organism from the CF sputum. This study shows that it has to be considered as having a potential clinical impact.